

Hepatotoxicity in Indigenous Sheep of Malaysia Stall-fed with Different Forms of *Brachiaria Decumbens*

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ABSTRAK

Lapan belas ekor biri-biri baka tempatan telah dibahagikan kepada tiga kumpulan perlakuan. Biri-biri ini diberi makan secukupnya di kadang sama ada dengan (I) rumput kering (hay), *Brachiaria decumbens* (II) rumput hijau *B. decumbens* atau (III) rumput *B. decumbens* kering dan mati yang telah dicampurkan dengan kek kelapa dan molases. Percubaan ini dijalankan dengan tujuan untuk menentukan sama ada bentuk pemberian yang berlainan ini boleh menyebabkan kehepatotoksikan. Selepas dua minggu, lima daripada enam ekor biri-biri dalam tiap-tiap kumpulan I dan II menunjukkan pelbagai darjah jaundis dan sedikit pemfotopekaan. Paras enzim SGOT dan SGGT juga didapati meningkat dengan ketara, menunjukkan kerosakan hati. Kerosakan hati pada biri-biri yang diberi makan rumput kering berlaku lebih awal daripada biri-biri yang diberi *B. decumbens* hijau. Percubaan ini telah menunjukkan bahawa pemberian rumput *B. decumbens* kepada biri-biri sama ada dalam bentuk rumput hijau atau rumput kering mungkin menyebabkan kehepatotoksikan.

ABSTRACT

Eighteen Indigenous Sheep were assigned to three treatment groups. They were stall-fed ad libitum with either (I) hay (II) green grass or (III) litter of *Brachiaria decumbens* mixed with copra cakes and molasses. The trial was conducted to determine whether hepatotoxicity will occur with the above method of feeding. After two weeks, five of six sheep in each group, I and II showed varying degrees of jaundice and mild photosensitization. Marked elevation of SGOT and SGGT concentration were observed in these animals, indicating liver damage. Liver damage occurred much earlier in sheep on hay than those fed green *B. decumbens*. Hence, this trial suggests that total feeding of *B. decumbens* to sheep either in the form of green grass or hay may cause hepatotoxicity.

INTRODUCTION

As early as 1979, cases of hemolytic anaemia and photosensitization-jaundice syndrome were reported to be responsible for high mortalities in sheep and goats in various farms in the country. The causative agent of the syndrome has not been conclusively identified.

Field cases occurring in the goat and sheep farm in MARDI* and also in the Department of Veterinary Services Goat and Sheep Breeding

stations in Gajah Mati, Kedah, indicated that the syndrome was closely associated with animals grazing on *Brachiaria decumbens*. Previous studies suggested that *Brachiaria* or *Brachiaria*-associated agents appear to be the predisposing factor which caused liver damage giving rise to the above syndrome (Abas Mazni *et al.*, 1983a). Similar symptoms were observed in sheep grazing continuously on pure stands of leafy regrowth of *B. decumbens* (Abas Mazni *et al.*, 1983b). Goats grazing continuously on *B. decumbens* showed a similar hepatotoxic syndrome to that seen in sheep. However, the effects on goats was slower and less severe

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(Abas Mazni *et al.*, 1985). In these studies, *Pithomyces chartarum*, a saprophytic fungus which was suggested to be associated with the hepatotoxic syndrome (Nobre and Andrade, 1976 and Carmago *et al.*, 1976) was not isolated. Similarly, copper was not found to be a primary factor causing the toxic liver damage (Abas Mazni *et al.*, 1983a). Thus these studies suggest that *B. decumbens* may contain a yet unidentified component which causes hepatotoxicity and photosensitization in sheep and goats.

Having recognised that continuous grazing of *B. decumbens* can cause a hepatotoxic syndrome in sheep, the objective of this study was to investigate the effect of stall feeding hay, green grass and litter of *B. decumbens* on the liver of indigenous sheep of Malaysia.

MATERIALS AND METHODS

Eighteen, healthy female indigenous sheep of Malaysia (ISM), ranging in age from 2 to 3 years were used in this experiment.

These animals were assigned to three treatment groups designated as I, II and III, with six animals in each group. Animals in group I were totally fed *Brachiaria* hay. The hay, made mechanically, was obtained from the Department of Veterinary Services (DVS) farm in Ijok, Selangor. Group II was totally fed green leafy *B. decumbens*, while group III was fed litter of dried and dead *B. decumbens* grass mixed with copra cakes (300 g/animal) and supplemented with molasses (200 ml) to increase palatability. The animals were fed *ad libitum*.

The animals were observed daily for changes in body condition, abnormal gait and movement, evidence of anemia and jaundice, signs of photosensitization and alterations of behavioural pattern. Live-weights were recorded at the beginning of the trial, at weekly intervals and at the end of the trial.

Two blood samples were taken weekly from the jugular vein of each animal. The first blood sample was taken in vacutainers containing EDTA. The blood was analysed for red blood cell count

(RBC), and haemoglobin concentration (Hb) using the Coulter ZF6 system^a while the packed cell volume (PCV) was determined using a haematocrit centrifuge^b. The plasma obtained from the haematocrit capillary tube was analysed for plasma protein using a refractometer^c. Haemoparasite examination was made on Giemsa stained blood smears. The second blood sample was taken in plain blood tubes and the separated serum was immediately analysed for serum glutamic-oxaloacetic transaminase (SGOT) and serum gamma-glutamyl transferase (SGGT) using the Roche Diagnostic kits^d.

Fungus isolation was performed by the methods recommended by the Ministry of Agriculture and Fisheries, New Zealand (Chapman and Di Menna, 1981) and Thornton and Sinclair (1960).

All dead animals were necropsied at the Faculty of Veterinary Medicine and Animal Science, Universiti Pertanian Malaysia.

RESULTS AND DISCUSSION

Ten sheep, five each from groups I and II, showed varying degrees of jaundice as early as two weeks on their respective diets. Mild photosensitization was observed in three sheep fed *Brachiaria* hay and four in those fed green *Brachiaria*. Lacrimation was also observed in most of the sheep with jaundice. Affected animals preferred to stay in the corner and showed loss of appetite. Eventually these animals lost weight and showed signs of dehydration. No significant change in the hematological picture was seen.

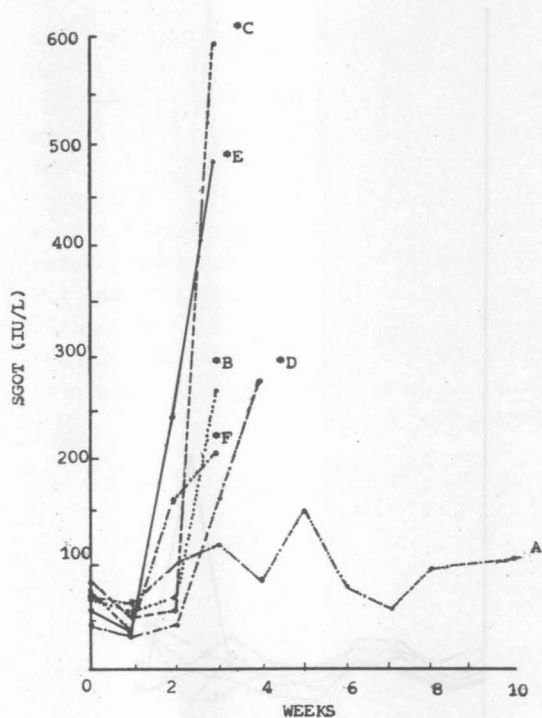
The photosensitization seen in this study was not as severe as that reported in earlier studies (Abas Mazni *et al.*, 1983a and b). This was probably due to the fact that the animals in this study were stallfed and therefore not exposed to direct sunlight. In the present study, the photosensitization may be the result of skin accumulation of phylloerythrin, (the end product of chlorophyll metabolism in the body) due to failure in its biliary excretion. Dermatitis develops when the sensitized skin is exposed to strong light.

^aCoulter Electronics Limited, London

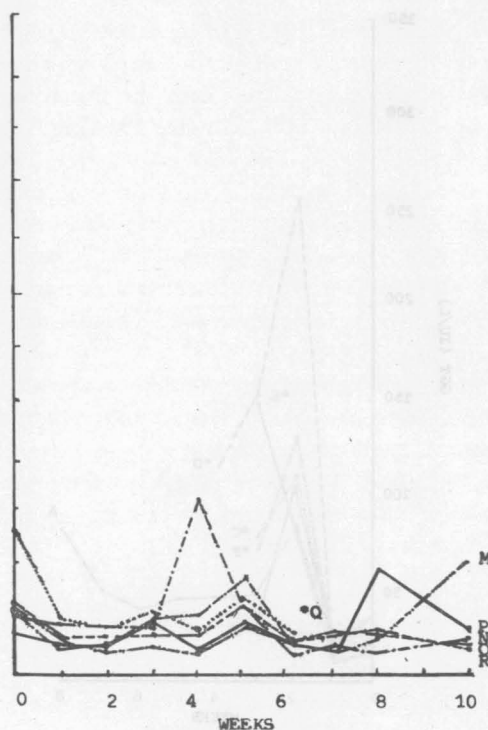
^bHawksley, England

^cBellingham and Stanley Limited, England

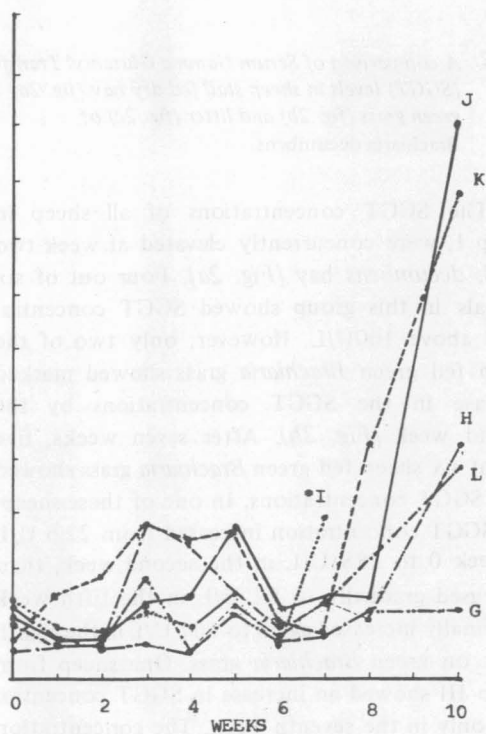
^dF. Hoffmann - La Roche & Co. Ltd.
Diagnostica, Basle, Switzerland



(a)



(c)

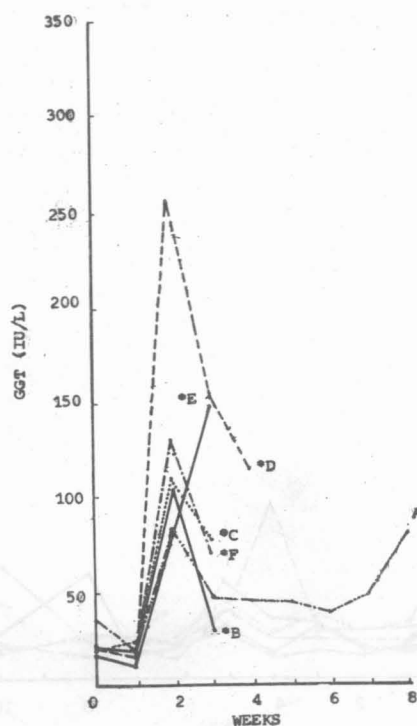


(b)

Fig. 1. A comparison of Serum - Glutamic Oxaloacetic Transaminase (SGOT) levels in sheep stall fed dry hay (Fig. 1a), green grass (Fig. 1b) and litter (Fig. 1c) of *Brachiaria decumbens*.

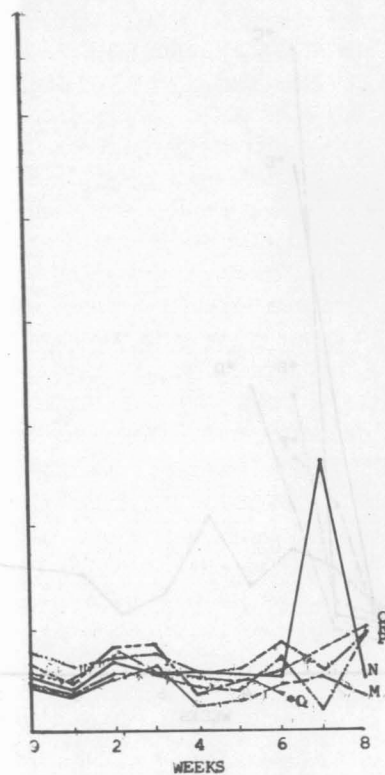
Three of the animals fed on *Brachiaria* hay diet for two weeks (group I), showed SGOT concentrations above 100 U/L. By three to four weeks the SGOT concentrations of five sheep in this group increased markedly to values ranging from 208 to 628 U/L (Fig. 1a). Similarly in the group of sheep fed green *Brachiaria* grass, variable increases in SGOT concentrations were observed initially.

Marked elevations in concentration of this enzyme were observed in four animals, only after the seventh week (Fig. 1b). The concentrations ranged from 194 to 529 U/L. However, there was no observable increased concentration of this enzyme in those animals fed litter of the grass (Fig. 1c).



*indicates death of animal.

(a)



(c)

Fig. 2: A comparison of Serum Gamma Glutamyl Transferase (SGGT) levels in sheep stall fed dry hay (fig. 2a) green grass (fig. 2b) and litter (fig. 2c) of *Brachiaria decumbens*.

The SGGT concentrations of all sheep in group I, were concurrently elevated at week two on *B. decumbens* hay (Fig. 2a). Four out of six animals in this group showed SGGT concentrations above 100U/L. However, only two of the sheep fed green *Brachiaria* grass showed marked increase in the SGGT concentrations by the second week (Fig. 2b). After seven weeks, five out of six sheep fed green *Brachiaria* grass showed high SGGT concentrations. In one of these sheep, the SGGT concentration increased from 22.6 U/L at week 0 to 288 U/L in the second week, then decreased gradually to 52 U/L in the fifth week and finally increased again to 307 U/L in the eighth week on green *Brachiaria* grass. One sheep from group III showed an increase in SGGT concentration only in the seventh week. The concentration of this enzyme subsequently returned to normal by the eighth week (Fig. 2c).

In sheep fed *Brachiaria* as hay or the fresh green form, the SGOT concentrations increased up to five-fold. The increase in SGOT coupled with the increase in SGGT concentrations in these sheep, suggest liver damage. Seven animals, give in group I, one each from group II and III died and were necropsied. Changes, similar to that in previous studies (Abas Mazni *et al.*, 1983a; 1983b) particularly hepatonecrosis, were observed. The present study indicates that liver damage occurred much earlier in sheep on *Brachiaria* hay than those fed green *Brachiaria* grass. The accelerated effect can be postulated to be due to the hepatotoxic substance in *B. decumbens* being more concentrated with the drying of the grass.

In this study, *Pithomyces chartarum* was not isolated in any of the treatment groups.

The findings indicate that the yet to be identified hepatotoxic factor that is present in the plant was neither destroyed nor its activity reduced in the process of sun drying. The toxic effect may either be the result of direct action of these toxic factors or its metabolite (s). Further work is therefore needed to identify the toxic principle in the plant and its mode of action. Our study as well as the previous studies (Abas Mazni *et al.*, 1983a; 1983b and 1985) indicate that attempts to find an alternative method of utilizing *B. decumbens* for feeding, especially to sheep may be futile at least until the nature of the toxic principle is known. The immediate solution should be towards discontinuing total feeding of *B. decumbens* to sheep and goats.

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